than 1,000 $TCID_{50}$ of virus, shall be excluded from the test. The geometric mean titer of antibody induced in the monkeys surviving the course of immunization and bleeding, shall be calculated. A comparison of the value so obtained shall be made with the value for the reference serum that was tested simultaneously and expressed as the ratio between the geometric mean titer value of the serums under test and the mean titer value of the reference serum.

(e) *Potency requirements.* A lot of vaccine tested against the reference serum shall be satisfactory if the geometric mean value of the group of individual monkey serums representing the lot of vaccine tested is at least 1.29 times the mean value of the reference serum for Type 1, at least 1.13 times for Type 2, and at least 0.72 times for Type 3.

[38 FR 32068, Nov. 20, 1973, as amended at 49 FR 23834, June 8, 1984; 50 FR 4137, Jan. 29, 1985; 55 FR 11013, Mar. 26, 1990]

§ 630.4 Tests for safety.

In the manufacture of the product, the following tests relating to safety shall be conducted by the manufacturer.

(a) *The virus pool—tests prior to inactivation*—(1) *B virus and Mycobacterium tuberculosis.* Prior to inactivation, each individual virus harvest or virus pool shall be tested for the presence of B virus and Mycobacterium tuberculosis.

(2) *SV–40.* Prior to inactivation, the material shall be tested for the presence of SV–40 as follows (or by any other test producing equally reliable results): A sample of at least 5 ml. from the virus harvest or virus pool shall be neutralized by high titer specific antiserum of other than primate origin. A similar sample from the pool of tissue culture fluids from control vessels representing the tissue from which the virus was prepared may be tested in place of the virus sample. The sample shall be tested in primary cercopithecus tissue cultures or in a cell line demonstrated as at least equally susceptible to SV–40. Each tissue culture system shall be observed for at least 14 days and at the end of the observation period at least one subculture of fluid shall be made in the same tissue culture system and the subculture shall be observed for at least 14 days.

(3) *Test results.* The virus harvest or virus pool is satisfactory for poliovirus vaccine only if the tests produce no evidence of the presence of B virus, Mycobacterium tuberculosis or SV–40.

(b) *Single strain pool tissue culture tests for poliovirus.* (1) Before pooling to make the final poliovirus vaccine, during inactivation at 36° to 38° C., two samples of each monovalent bulk strain pool shall be tested for the presence of virus by tissue culture methods, the second sample to be taken at least 3 days after taking the first sample.

(2) Each sample shall be no smaller than the equivalent of 1,500 human doses and shall be subjected to the complete testing process and each test shall be performed on a different monkey kidney tissue culture cell preparation. The test sample for one of these tests may be used also for the test prescribed in paragraph (f) of this section provided the cell cultures used have been demonstrated as fully susceptible to SV–40 and poliovirus. Each sample shall be inoculated into five or more tissue culture bottles of a suitable capacity, the ratio of the vaccine to the nutrient fluid being approximately 1:1 to 1:3, and the area of the surface growth of cells being at least 3 square centimeters per milliliter of sample. The tissue culture bottles shall be observed for at least 14 days.

(3) A first subculture shall be made at the end of 7 days from date of inoculation by planting at least 2 percent of the volume from each original bottle into suitable tissue culture vessels, followed by refeeding.

(4) A second subculture shall be made from each original bottle in the same manner at the end of 14 days from date of inoculation.

(5) Each of the first and second subcultures shall be observed for at least 7 days.

(6) If cytopathogenic effects occur either in the original bottles of the two tests or in the subcultures from them, or if cellular degeneration appears in the original bottles or in the subcultures before degeneration occurs in uninoculated cultures, the pool shall be

held until the matter is resolved. If active poliovirus is indicated, the strain pool shall not be used for inclusion in a final vaccine unless effectively reprocessed as described in §630.2(e). If other viruses are present, the pool shall not be used unless it can be demonstrated that such viruses have originated from other than the strain pool being tested.

(c) *Trivalent vaccine pool tissue culture test.* No less than 1,500 human doses of the trivalent vaccine pool, without final preservative, prepared by pooling the three type pools, each of which has passed all tests prescribed in paragraph (b) of this section, shall be subjected to the complete tissue culture test prescribed in such paragraph (b) in at least two approximately equal tests in separate monkey kidney tissue culture preparations. This test sample may be used also for the test prescribed in paragraph (f) of this section provided the cell cultures used have been demonstrated as fully susceptible to SV–40 and poliovirus.

(d) *Trivalent vaccine pool lymphocytic choriomeningitis test.* The final vaccine shall be shown to be free of lymphocytic choriomeningitis virus by intracerebral inoculation of the maximum volume tolerated into 10 or more mice which shall be observed daily for at least 21 days and a negative test shall not be valid unless at least eight mice survive for this period.

(e) *Test in monkeys for active virus.* (1) Vaccine from final containers selected at random from each filling of each lot shall be pooled to provide a test sample of at least 400 milliliters representing the various fillings. An equal volume of bulk vaccine may be substituted for test samples from each filling lot provided the procedure has been approved by the Director, Center for Biologics Evaluation and Research.

(2) A total of not less than 20 monkeys shall be inoculated with the test sample. A preinjection serum sample from each monkey must not contain neutralizing antibody against the three poliovirus types detectable in a dilution of 1:4 when tested against not more than 1,000 $TCID_{50}$ of virus. At least 80 percent of the test animals representing each filling or each bulk sample must survive the test period without significant weight loss, except that if at least 60 percent of the test animals survive the first 48 hours after injection, those animals which do not survive this 48-hour test period may be replaced by an equal number of test animals. At least 80 percent of the animals used in the test must show microscopic evidence of inoculation trauma in the lumbar region of the spinal cord, and gross or microscopic evidence of inoculation trauma in the thalamic area. If less than 60 percent of the test animals survive the first 48 hours, or if less than 80 percent of the animals fail to meet the other criteria prescribed in this section, the test must be repeated.

(3) Vaccines shall be injected by combined intracerebral, intraspinal, and intramuscular routes into Macaca or Cercopithecus monkeys or a species found by the Director, Center for Biologics Evaluation and Research, to be equally suitable for the purpose. The animals shall be in overt good health and injected under deep barbiturate anesthesia. The intracerebral injection shall consist of 0.5 milliliter of test sample into the thalamic region of each hemisphere. The intraspinal injection shall consist of 0.5 milliliter of concentrated test sample into the lumbar spinal cord enlargement, the test sample to be concentrated 100 fold in the ultracentrifuge by a method demonstrated to recover at least 90 percent of the virus particles in the sediment after it has been resuspended in the same lot of unconcentrated test sample. The intramuscular injection shall consist of 1.0 milliliter of test sample into the right leg muscles. At the same time, 200 milligrams of cortisone acetate shall be injected into the left leg muscles, and 1.0 milliliter of procaine penicillin (300,000 units) into the right arm muscles. The monkeys shall be observed for 17 to 19 days and signs suggestive of poliomyelitis shall be recorded.

(4) At the end of the observation period, samples of cerebral cortex and of cervical and lumbar spinal cord enlargements shall be taken for virus recovery and identification. Histological sections shall be prepared from both spinal cord enlargements and examined.

(5) Doubtful histopathological findings necessitate (i) examination of a sample of sections from several regions of the brain in question, and (ii) attempts at virus recovery from the nervous tissues previously removed from the animal. The test results must be negative. Test results are negative if the histological and other studies leave no doubt that poliovirus infection did not occur.

(f) *Tissue culture safety test for SV–40.* At least 500 human doses of each monovalent or trivalent pool of vaccine shall be tested for the presence of SV–40 using primary cercopithecus monkey tissue cultures or using a cell line demonstrated as at least equally susceptible to SV–40. The test shall be conducted as described in paragraph (b) of this section, except for the volume of test sample and except that one subculture of at least 2 percent of the volume of the fluids shall be made no less than 14 days from the date of inoculation and examined for at least 14 days from the date of subinoculation. The vaccine is satisfactory only if there is no evidence of the presence of SV–40 in any of the cultures or subcultures.

[38 FR 32068, Nov. 20, 1973, as amended at 49 FR 23834, June 8, 1984; 50 FR 4137, Jan. 29, 1985; 50 FR 16229, Apr. 25, 1985; 55 FR 11013, Mar. 26, 1990; 57 FR 10814, Mar. 31, 1992]

§ 630.5 General requirements.

(a) *Consistency of manufacture.* No lot of final vaccine shall be released unless it is one of a series of five consecutive lots produced by the same manufacturing process, all of which have shown negative results with respect to all tests for the presence of live poliovirus, and unless each of the monovalent pools of which a polyvalent final vaccine is composed similarly is one of a series of five consecutive monovalent pools of the same type of inactivated poliovirus, all of which have shown negative results in all tests for the presence of live poliovirus.

(b) *Dose.* These additional standards are based on a human dose of 1.0 milliliter for a single injection and a total human immunizing dose of three injections of 1.0 milliliter given at appropriate intervals.

(c) *Samples and protocols.* For each lot of vaccine, the following material shall be submitted to the Director, Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville Pike, Bethesda, MD 20892:

(1) A 2,500 milliliter sample, neutralized, not dialyzed, and without final preservative, taken at the latest possible stage of manufacturing before the addition of such preservative.

(2) A 200 milliliter bulk sample of the final vaccine containing final preservative.

(3) A total of not less than a 200 milliliter sample of the final vaccine in final labeled containers.

(4) A protocol which consists of a summary of the history of manufacture of each lot including all results of each test for which test results are requested by the Director, Center for Biologics Evaluation and Research.

[38 FR 32068, Nov. 20, 1973, as amended at 49 FR 23834, June 8, 1984; 51 FR 18580, May 21, 1986; 55 FR 11013, Mar. 26, 1990]

Subpart B—Poliovirus Vaccine Live Oral Trivalent

SOURCE: 56 FR 21432, May 8, 1991, unless otherwise noted.

§ 630.10 Poliovirus Vaccine Live Oral Trivalent.

(a) *Proper name and definition.* The proper name of this product shall be Poliovirus Vaccine Live Oral Trivalent. The vaccine shall be a preparation containing the three types of live, attenuated polioviruses grown in monkey kidney cell cultures, or in a cell line found by the Director, Center for Biologics Evaluation and Research, to meet the requirements of §610.18(c) of this chapter. The vaccine shall be prepared in a form suitable for oral administration.

(b) *Criteria for acceptable strains.* (1) The Sabin strains of attenuated poliovirus, Type 1 (LS-c, 2ab/KP_2), Type 2 (P712, Ch, 2ab/KP_2), and Type 3 (Leon $12a_1b/KP_3$), or derivatives from them, may be used in the manufacture of vaccine.

(2)(i) Other poliovirus strains may be used in the manufacture of Poliovirus Vaccine Live Oral Trivalent provided that they are identified by historical records including:

(A) Origin,